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Hydration Structure around the Methylene Group of Glycine Molecule

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Neutron diffraction measurements have been carried out on aqueous 4 mol% glycine solutions. The H/D isotopic substitution technique was applied to both methylene-hydrogen (H_M) and water-hydrogen (H_W) atoms in order to obtain information concerning the hydration structure around the methylene group within the glycine molecule. Structural parameters for the first hydration shell of the methylene-hydrogen atoms were determined as $r(H_M\cdots O_W) = 2.64(1)$ Å and $r(H_M\cdots H_W) = 2.87(1)$ Å from the least squares fitting analysis of the observed first-order difference function, ${}^{0H}\Delta_H(Q)$, and the partial structure factor, $a_{H_MH_{ex}}(Q)$, respectively. The first hydration shell of the methylene group involves ca. 2 water molecules per one glycine molecule.

Structural properties of hydrated amino acid molecules in aqueous solutions have long been a matter of interest for extensive fields of chemical and biological sciences. Since the amino acid molecule is "poly-functional", it is necessary to elucidate the hydration structure around each functional group to discuss the solvation structure of the amino acid molecule as a whole.[1] A Monte Carlo computer simulation study by Mezei et al. has shown that numbers of water molecules around the amino, methylene, and carboxyl groups of a zwitterionic glycine molecule ($NH_3{}^+CH_2COO^-$) in aqueous solution, are 3.2, 6.1, and 5.1, respectively.[2] The hydration structure of the amino group within the glycine molecule in the neutral aqueous solution has recently been investigated by neutron diffraction with ${}^{14}N/{}^{15}N$ and H/D isotopic substitution techniques.[3] The result showed that 3.0(6) water molecules are hydrogen-bonded to the amino group with intermolecular distances of $r_{N\cdots O} = 2.85(5)$ Å and $r_{N\cdots H} = 3.25(5)$ Å.[3] More recently, neutron diffraction investigations have conducted in order to determine the hydration structure of the glycine molecule under high-[4] and low-pH[5] conditions. In alkaline solution, it was discovered that the amino group forms one hydrogen bond of the N···D–OD type ($r_{N\cdots D} = 1.97(3)$ Å), and simultaneously forms bonds of the N–D···OD_2 type with ca. 2 water molecules ($r_{N\cdots O} = 2.92(3)$ Å).[4] The amino group of the glycine molecule in the acidic solution was found to form hydrogen bonds of the N···OD_2 type with ca. 3 water molecules ($r_{N\cdots O} = 2.90(2)$ Å).[5] These results indicate that the hydration structure around the amino group is strongly pH-dependent. The hydration structure around the methylene group in the alkaline and acidic solutions has been examined by neutron diffraction using the H/D isotopic substitution technique.[4,5] The observed intermolecular distribution function around the methylene-hydrogen atom, $G_H{}^{\mathrm{inter}}(r)$, exhibits a partially resolved first peak at $r = 2.6$ and 2.7 Å for the alkaline and the acidic solutions, respectively. This implies that the distribution of water molecules neighboring the methylene-hydrogens is not random, i.e., the methylene group of the glycine molecule has the first hydration shell. The average intermolecular distances between the methylene-hydrogen atom and the nearest neighbor water molecule in the alkaline and acidic solutions have been reported to be $r_{H_M\cdots D_2O} = 2.63(1)$ Å[4] and 2.68(1) Å,[5] respectively. The coordination number around a methylene-hydrogen atom in the alkaline and acidic solutions has been observed to be 0.81(6)[4] and 0.66(1),[5] respectively. In order to obtain information on the orientational correlation of water molecules in the first hydration shell of the methylene group, the partial structure factors $a_{H_MO_W}(Q)$ (H_M: methylene-hydrogen, O_W: water-oxygen) and $a_{H_MH_W}(Q)$ (H_W: water-hydrogen) have to be determined.

In the present paper, we describe the results of neutron diffraction measurements on four aqueous 4 mol% glycine solutions in which the H/D isotopic ratios of both the methylene-hydrogen and water-hydrogen atoms have been changed. The details of the hydration structure around the methylene-hydrogen atoms are deduced from observed partial structure factors, $a_{H_MH_{ex}}(Q)$ (H_{ex}: exchangeable hydrogen atom) and $a_{H_MX}(Q)$ (X: O, C, N, and H_M), which are obtained from the combination of the observed difference functions, ${}^{D}\Delta_H(Q)$, between $(ND_2CD_2COOD)_{0.04}(D_2O)_{0.96}$ and $(ND_2CH_2COOD)_{0.04}(D_2O)_{0.96}$, and ${}^{0H}\Delta_H(Q)$, between $(N^0H_2CD_2COO^0H)_{0.04}({}^0H_2O)_{0.96}$ and $(N^0H_2CH_2COO^0H)_{0.04}({}^0H_2O)_{0.96}$ solutions (${}^0H$: H/D = 64/36, $b_{H_{ex}} = 0$), respectively.

Experimental

Materials. Exchangeable hydrogen atoms within natural NH_2CH_2COOH (Nacalai Tesque, guaranteed grade) were deuterated by dissolving them into 10 times the molar quantity of D_2O (99.9% D, Aldrich Chemical Co., Inc.), followed by dehydration under vacuum. This procedure was repeated 3 times. The D-enriched ND_2CH_2COOD and ND_2CD_2COOD (98.0% D, ISOTEC Inc.) were dissolved into D_2O (99.9% D, Aldrich Chemical Co., Inc.) and into a H_2O–D_2O mixture to prepare four kinds of aqueous 4 mol% glycine solutions with different isotopic compositions of both the methylene hydrogens and exchangeable hydrogen atoms, i.e.,

I: $(ND_2CD_2COOD)_{0.04}(D_2O)_{0.96}$,
II: $(ND_2CH_2COOD)_{0.04}(D_2O)_{0.96}$,
III: $(N^0H_2CD_2COO^0H)_{0.04}({}^0H_2O)_{0.96}$, and
IV: $(N^0H_2CH_2COO^0H)_{0.04}({}^0H_2O)_{0.96}$, respectively.

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Table 1. Isotopic Compositions and Mean Scattering Lengths, $b_{\mathrm{H_M}}$ and $b_{\mathrm{H_{ex}}}$ of Methylene- and Exchangeable-Hydrogen Atoms, Total Cross Sections, and Number Density Scaled in the Stoichiometric Unit, $(\mathrm{N^*H_2C^*H_2COO^*H})_{0.04}(\mathrm{^*H_2O})_{0.96}$, σ_t and ρ, Respectively

	Sample	$\mathrm{H_M}$ /%a)	$\mathrm{D_M}$ /%a)	$\mathrm{H_{ex}}$ /%b)	$\mathrm{D_{ex}}$ /%b)	$b_{\mathrm{H_M}}$ /10^{-12} cm	$b_{\mathrm{H_{ex}}}$ /10^{-12} cm	σ_t /barnsc)	ρ /Å^{-3}
Ⅰ	$(\mathrm{ND_2CD_2COOD})_{0.04}(\mathrm{D_2O})_{0.96}$	2.0	98.0	0.1	99.9	0.646	0.666	14.484	0.03146
Ⅱ	$(\mathrm{ND_2CH_2COOD})_{0.04}(\mathrm{D_2O})_{0.96}$	100	0	0.1	99.9	−0.374	0.666	16.879	
Ⅲ	$(\mathrm{N^0H_2CD_2COO^0H})_{0.04}(\mathrm{^0H_2O})_{0.96}$	2.0	98.0	64.1	35.9	0.646	0	55.372	
Ⅳ	$(\mathrm{N^0H_2CH_2COO^0H})_{0.04}(\mathrm{^0H_2O})_{0.96}$	100	0	64.1	35.9	−0.374	0	57.938	

a) For methylene-hydrogen atoms of glycine molecule. b) For exchangeable-hydrogen atoms. c) For incident neutron wavelength of 1.104 Å.

The H/D isotopic composition of the methylene-hydrogen atoms in the deuterated crystalline $\mathrm{ND_2CD_2COOD}$ was evaluated from the integral intensity of the diffuse reflectance infrared spectra of the first overtone of the C–H stretching vibrational band. The infrared spectra were obtained using a JASCO FT/IR-410 spectrometer with a DR-81 diffuse reflection attachment. The observed value of the D content, $98.0 \pm 0.6\%$, is in good agreement with that quoted in the manufacturer's specification. The isotopic ratio of the exchangeable hydrogen atoms ($\mathrm{H_{ex}}$) in the sample solutions Ⅲ and Ⅳ (H/D = 64/36), was chosen to make the average coherent scattering length of $\mathrm{H_{ex}}$ zero, implying that there is no contribution from $\mathrm{H_{ex}}\cdots\mathrm{j}$ pairs in the observed interference term. The sample solution was sealed into a cylindrical quartz cell (11.4 mm in inner diameter and 1.2 mm in thickness). The coherent scattering lengths and scattering absorption cross sections for O and N nuclei were derived from the corresponding values tabulated by Sears.[6] The scattering cross sections for H and D nuclei calculated from the observed total cross sections for liquid $\mathrm{H_2O}$ and $\mathrm{D_2O}$[7] were applied to the absorption correction of diffraction intensities. The sample parameters are summarized in Table 1.

Neutron Diffraction Measurements. The neutron diffraction measurements were carried out at 25 °C using the ISSP 4G (GPTAS) diffractometer installed at the JRR-3M research reactor operated at 20 MW, in The Japan Atomic Energy Research Institute in Tokai, Japan. The incident neutron wavelength, $\lambda = 1.104 \pm 0.005$ Å, was determined from Bragg reflections from the KCl powder. Collimations used were 40′–80′–80′ in going from the reactor to the detector. The aperture of the collimated beam was 20 mm in width and 40 mm in height. Scattered neutrons were collected over the angular range of $3.0 \le 2\theta \le 115°$, corresponding to the scattering vector magnitude range of $0.30 \le Q \le 9.60$ Å^{-1} ($Q = 4\pi \sin\theta/\lambda$). The step interval was chosen to be $\Delta(2\theta) = 0.5°$ in the range of $3 \le 2\theta \le 40°$, and $\Delta(2\theta) = 1°$ in the range of $41 \le 2\theta \le 115°$, respectively. The preset neutron monitor count was 1000×10^6 for samples Ⅰ and Ⅱ, and 1500×10^6 for samples Ⅲ and Ⅳ. Scattering intensities from the $\mathrm{^0H_2O}$ solution were measured for the inelasticity correction of observed difference functions, which will be discussed later. Measurements of scattering intensities from the vanadium rod (8 mm in diameter), empty cell, and background were made in advance.

Data Analysis. The measured scattering intensities were corrected for the instrumental background and absorption of both the sample and cell.[8] A multiple scattering correction was adopted for the observed scattering intensities from the vanadium rod.[9] The count rate of the sample was converted to the absolute scale by the use of corrected scattering intensities from the vanadium rod.

The first-order difference functions,[10,11] ${}^{\mathrm{D}}\Delta_{\mathrm{H}}(Q)$ and ${}^{0\mathrm{H}}\Delta_{\mathrm{H}}(Q)$, were obtained from the numerical difference between observed scattering cross sections of samples Ⅰ and Ⅱ, and between samples Ⅲ and Ⅳ, respectively,

$$
\begin{aligned}
{}^{\mathrm{D}}\Delta_{\mathrm{H}}(Q) &= (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{obs}}(\text{for sample Ⅰ}) \\
&\quad - (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{obs}}(\text{for sample Ⅱ}), \qquad (1)\\
{}^{0\mathrm{H}}\Delta_{\mathrm{H}}(Q) &= (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{obs}}(\text{for sample Ⅲ}) \\
&\quad - (\mathrm{d}\sigma/\mathrm{d}\Omega)^{\mathrm{obs}}(\text{for sample Ⅳ}). \qquad (2)
\end{aligned}
$$

$\Delta_{\mathrm{H}}(Q)$, scaled to stoichiometric units, $(\mathrm{N^*H_2C^*H_2COO^*H})_{0.04}(\mathrm{^*H_2O})_{0.96}$, can be represented as a linear combination of partial structure factors related to the methylene-hydrogen atoms:

$$
\begin{aligned}
\Delta_{\mathrm{H}}(Q) &= A[a_{\mathrm{H_MO}}(Q) - 1] + B[a_{\mathrm{H_MH_{ex}}}(Q) - 1] \\
&\quad + C[a_{\mathrm{H_MC}}(Q) - 1] \\
&\quad + D[a_{\mathrm{H_MN}}(Q) - 1] + E[a_{\mathrm{H_MH_M}}(Q) - 1], \qquad (3)
\end{aligned}
$$

where

$$
\begin{aligned}
&A = 4x(1+x)b_{\mathrm{O}}\Delta b_{\mathrm{H_M}}, \quad B = 4x(2+x)b_{\mathrm{H_{ex}}}\Delta b_{\mathrm{H_M}}, \\
&C = 8x^2 b_{\mathrm{C}}\Delta b_{\mathrm{H_M}}, \quad D = 4x^2 b_{\mathrm{N}}\Delta b_{\mathrm{H_M}}, \\
&\text{and} \quad E = 4x^2({b_{\mathrm{D_M}}}^2 - {b_{\mathrm{H_M}}}^2).
\end{aligned}
$$

$\Delta b_{\mathrm{H_M}}$ stands for the difference in the coherent scattering length of the methylene-hydrogen atoms between the two sample solutions. The methylene- and exchangeable-hydrogen atoms are denoted by $\mathrm{H_M}$ and $\mathrm{H_{ex}}$, respectively. Numerical values of the weighting factors in Eq. 3, $A - E$, are listed in Table 2. The distribution function around the methylene-hydrogen atoms, $G_{\mathrm{H}}(r)$, is derived from the Fourier transform of $\Delta_{\mathrm{H}}(Q)$:

$$
\begin{aligned}
G_{\mathrm{H}}(r) &= 1 + (A+B+C+D+E)^{-1}(2\pi^2\rho r)^{-1} \\
&\quad \times \int_0^{Q_{\max}} Q\Delta_{\mathrm{H}}(Q)\sin(Qr)\mathrm{d}Q \\
&= [Ag_{\mathrm{H_MO}}(r) + Bg_{\mathrm{H_MH_{ex}}}(r) + Cg_{\mathrm{H_MC}}(r) \\
&\quad + Dg_{\mathrm{H_MN}}(r) + Eg_{\mathrm{H_MH_M}}(r)] \\
&\quad \times (A+B+C+D+E)^{-1}. \qquad (4)
\end{aligned}
$$

The upper limit of the integral, $Q_{\max}$, was set to be 9.6 Å^{-1} in the present study.

The intramolecular $\mathrm{H_M}\cdots\alpha$ contribution within the glycine molecule, ${I_{\mathrm{H}}}^{\mathrm{intra}}(Q)$, described below, was evaluated by applying molecular parameters from single crystal X-ray,[12] Neutron,[12,13] and gas phase electron diffraction[14] studies:

$$
\begin{aligned}
{I_{\mathrm{H}}}^{\mathrm{intra}}(Q) &= \sum 2c_{\mathrm{H_M}} b_\alpha \Delta b_{\mathrm{H_M}} \exp(-{l_{\mathrm{H_M}\alpha}}^2 Q^2/2) \\
&\quad \times \sin(Qr_{\mathrm{H_M}\alpha})/(Qr_{\mathrm{H_M}\alpha}), \qquad (5)
\end{aligned}
$$

Table 2. Values of the Coefficients of $a_{ij}(Q)$ in Eq. 3

Difference function	A/barns	B/barns	C/barns	D/barns	E/barns
${}^{D}\Delta_H(Q)$	0.0986	0.2219	0.0087	0.0061	0.0018
${}^{0H}\Delta_H(Q)$	0.0986	0	0.0087	0.0061	0.0018

where c_{H_M} is the number of methylene-hydrogen atoms in stoichiometric units. Parameters $l_{H_M\alpha}$ and $r_{H_M\alpha}$ denote the root-mean-square amplitude and internuclear distance for the $H_M\cdots\alpha$ pair, respectively. $I_H{}^{intra}(Q)$ was then subtracted from the observed total $\Delta_H(Q)$ to obtain the intermolecular difference function, $\Delta_H{}^{inter}(Q)$:

$$\Delta_H{}^{inter}(Q) = \Delta_H(Q) - I_H{}^{intra}(Q). \tag{6}$$

The intermolecular distribution function, $G_H{}^{inter}(r)$, is obtained from the Fourier transform of $\Delta_H{}^{inter}(Q)$ using Eq. 4 with the upper limit of the integral, $Q_{max} = 9.6$ Å^{-1}.

The intermolecular H_M–H_{ex} partial structure factor $a_{H_MH_{ex}}(Q)$ is derived from the second order difference between the observed ${}^{D}\Delta_H(Q)$ and ${}^{0H}\Delta_H(Q)$:

$$\begin{aligned} {}^{D}\Delta_H(Q) - {}^{0H}\Delta_H(Q) = 4x(2-x)(b_{H_M} - b_{H_M}{}') \\ \times (b_{H_{ex}} - b_{H_{ex}}{}')[a_{H_MH_{ex}}(Q) - 1]. \end{aligned} \tag{7}$$

Since the contribution from the H_M–H_W (H_W: water-hydrogen atom) pair occupies ca. 94% of $a_{H_MH_{ex}}(Q)$ in the present experimental conditions, $a_{H_MH_{ex}}(Q)$ can be regarded as the distribution of the water-hydrogen atoms around the methylene-hydrogen atoms of the glycine molecule. The intermolecular difference function, ${}^{0H}\Delta_H(Q)$, is directly connected to the partial structure factor, $a_{H_MX}(Q)$ (X: O, C, N, and H_M):

$${}^{0H}\Delta_H(Q) = (A + C + D + E)[a_{H_MX}(Q) - 1]. \tag{8}$$

Since the contribution of the H_M–O_W (O_W: water-oxygen atom) pair is ca. 86% of $a_{H_MX}(Q)$ in the present experimental conditions, $a_{H_MX}(Q)$ can be well approximated as the interaction between the methylene-hydrogen and water-oxygen atoms. The intermolecular partial distribution function, $g_{H_Mj}(r)$ (j: H_{ex}, X), is obtained from the Fourier transform as follows:

$$g_{H_Mj}(r) = 1 + (2\pi^2\rho r)^{-1}\int_0^{Q_{max}} Q[a_{H_Mj}(Q) - 1]\sin(Qr)\mathrm{d}Q. \tag{9}$$

The upper limit of the integral is set to $Q_{max} = 9.6$ Å^{-1} in the present analysis.

Structural parameters concerning hydration around the methylene-hydrogen atom were determined through a least squares fitting analysis of the observed $a_{H_MH_{ex}}(Q)$ and $a_{H_MX}(Q)$ by employing the model function $a_{H_Mj}(Q)$, involving the short- and long-range contributions:[15–17]

$$\begin{aligned} a_{H_Mj}{}^{model}(Q) = \sum 2\beta_j n_{H_Mj}\exp(-l_{H_Mj}{}^2Q^2/2) \\ \times \sin(Qr_{H_Mj})/(Qr_{H_Mj}) \\ + 4\pi\rho\exp(-l_{0j}{}^2Q^2/2)[Qr_{0j}\cos(Qr_{0j}) \\ - \sin(Qr_{0j})]Q^{-3}, \end{aligned} \tag{10}$$

where, $\beta_{H_{ex}} = 2(1-x)/(2-x)$ and $\beta_X = A/(A + C + D + E)$. The fitting procedure was made in the range of $0.3 \le Q \le 9.6$ Å^{-1} using the SALS program,[18] with the assumption that the statistical uncertainties distribute uniformly.

Results and Discussion

Scattering cross sections, $(\mathrm{d}\sigma/\mathrm{d}\Omega)^{obs}$, observed for isotopically substituted aqueous 4 mol% glycine solutions are shown in Fig. 1. Interference features of the observed scattering cross sections for sample solutions with different H_M/D_M ratios look very similar. However, a systematic difference in the intensity can be identified around the first diffraction peak at $Q \approx 2$ Å^{-1}, which corresponds to the difference in the coherent scattering length of the methylene-hydrogen atoms. The decrease in $(\mathrm{d}\sigma/\mathrm{d}\Omega)^{obs}$ at the higher-Q region is obviously due to the inelasticity effect, which is significant for samples containing large amounts of H atoms.

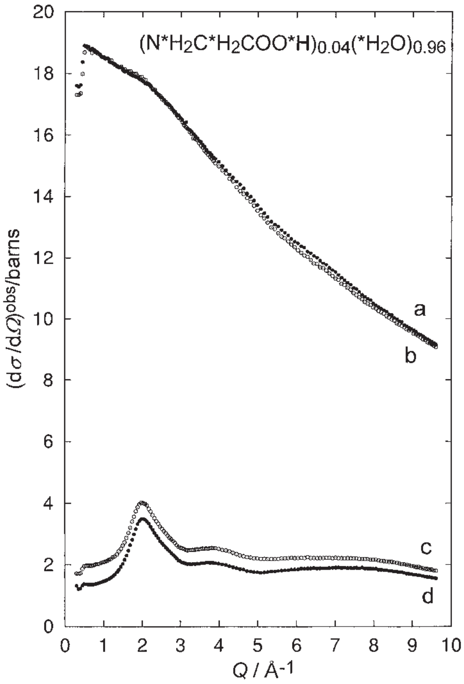

Fig. 1. Observed scattering cross sections $(\mathrm{d}\sigma/\mathrm{d}\Omega)^{obs}$ for aqueous 4 mol% glycine solutions with different isotopic ratios of H_M/D_M (H_M: methylene-hydrogen atom) and H_{ex}/D_{ex} (H_{ex}: exchangeable hydrogen atom). a) D_M–${}^{0}H_{ex}$. b) H_M–${}^{0}H_{ex}$. c) H_M–D_{ex}. d) D_M–D_{ex}. ${}^{0}H$ denotes the isotopic mixture in which the average scattering length is zero.

In order to deduce the hydration structure around the methylene-hydrogen atoms, difference functions, ${}^{D}\Delta_H(Q)$ and ${}^{0H}\Delta_H(Q)$, were calculated from the difference between observed scattering cross sections for D_2O solutions I–II and between ${}^{0}H_2O$ solutions, III–IV, respectively. In general, the inelasticity effect arising from H and D atoms contained in the solvent is subtracted out in the deduced first-order difference function. However, in the H/D substitution experiment, the inelasticity contribution remains in the observed difference function, $\Delta_H(Q)$.[19] Since the magnitude of the inelasticity distortion in the observed self scattering term is much larger for the H nucleus than for the other nuclei, the inelasticity distortion is considered to be approximately proportional to the number of H atoms involved in the sample solution. In the present study, we adopted the inelasticity correction procedure using the self scattering intensity observed for liquid ${}^0H_2O$. The self term of the ${}^0H_2O$ was derived by subtracting the calculated O–O partial structure factor, $a_{OO}(Q)$, from the observed scattering cross section of ${}^0H_2O$:

$$\begin{aligned}&(d\sigma/d\Omega)^{self}(\text{for } {}^0H_2O)\\&\quad = (d\sigma/d\Omega)^{obs}(\text{for } {}^0H_2O) - b_O{}^2[a_{OO}(Q) - 1].\end{aligned} \qquad (11)$$

The $a_{OO}(Q)$ for water was evaluated using the short- and the long-range structure parameters determined in our previous neutron diffraction study.[20] We adopted the inelasticity correction for the observed first-order difference function, $\Delta_H(Q)^{obs}$, to give the following equation:

$$\begin{aligned}\Delta_H(Q) &= \Delta_H{}^{obs}(Q) - (N_H{}^{\Delta}/N_H{}^{{}^0H_2O})\\&\quad \times (d\sigma/d\Omega)^{obs}(\text{for } {}^0H_2O).\end{aligned} \qquad (12)$$

Here, $N_H{}^{\Delta}$ and $N_H{}^{{}^0H_2O}$ denote the number of methylene- and water-H atoms involved in stoichiometric units, $(N^*H_2C^*H_2COO^*H)_{0.04}({}^*H_2O)_{0.96}$ and ${}^0H_2O$.

The observed difference function, ${}^D\Delta_H(Q)$, and the corresponding distribution function around the methylene-hydrogen atoms within the glycine molecule, ${}^DG_H(r)$, are represented in Figs. 2 and 3, respectively. In Fig. 2a, the observed ${}^D\Delta_H(Q)$ function exhibits an oscillatory feature extending to the higher-Q region. The overall normalization error in the observed ${}^D\Delta_H(Q)$ function was estimated by a least squares fit of the calculated intramolecular contribution, $I_H{}^{intra}(Q)$, in the sufficiently high-Q region. The normalization factor, γ, defined by $I_H{}^{intra}(Q) = \gamma \times {}^D\Delta_H(Q)$, was obtained to be $\gamma = 0.94 \pm 0.02$ from the least squares fit in the range of $4.0 \le Q \le 9.6$ Å^{-1}. This result implies that the present data correction and normalization procedures have been adequately carried out. The overall normalization error in the present ${}^D\Delta_H(Q)$ function is estimated to be within ca. 6%. The calculated $I_H{}^{intra}(Q)$ was then subtracted from ${}^D\Delta_H(Q)$ to obtain the intermolecular difference function, ${}^D\Delta_H{}^{inter}(Q)$, which is represented in Fig. 2c. The ${}^D\Delta_H{}^{inter}(Q)$ function is characterized by the first peak located at $Q \approx 2$ Å^{-1}.

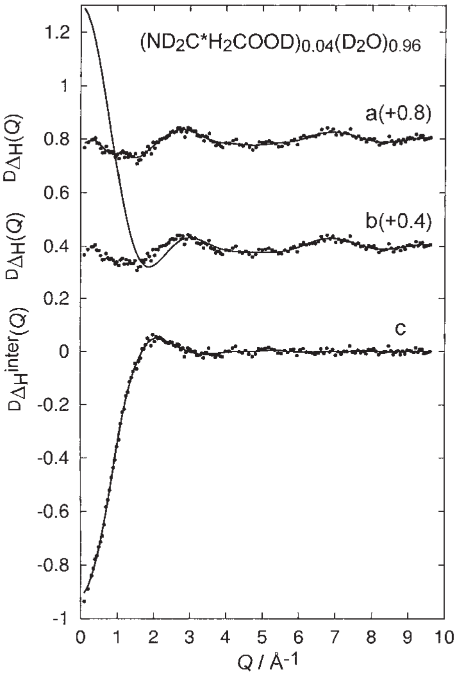

Fig. 2. a) Dots: The observed difference function, ${}^D\Delta_H(Q)$, for 4 mol% glycine solution in D_2O. The solid line: The back-Fourier transform of ${}^DG_H(r)$ shown by the solid line in Fig. 3a. b) Dots: The observed ${}^D\Delta_H(Q)$. The solid line: The intramolecular contribution within the glycine molecule, $I_H{}^{intra}(Q)$. c) Dots: The intermolecular difference function, ${}^D\Delta_H{}^{inter}(Q)$. The solid line: The back-Fourier transform of ${}^DG_H{}^{inter}(r)$ indicated by the solid line in Fig. 3b.

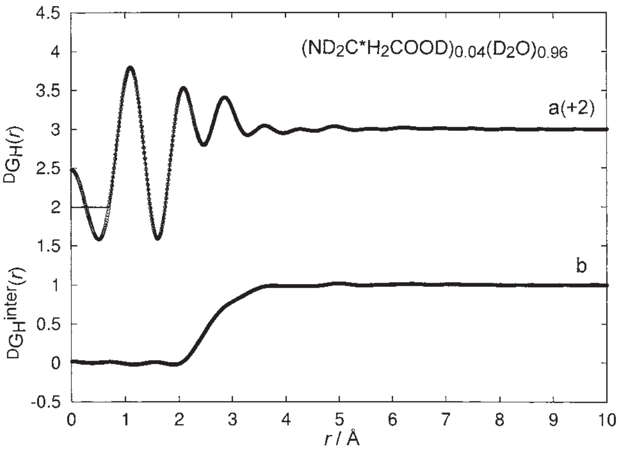

Fig. 3. a) The total and b) intermolecular distribution function around the methylene-hydrogen atom of the glycine molecule, ${}^DG_H(r)$ and ${}^DG_H{}^{inter}(r)$, respectively, observed for the aqueous 4 mol% glycine solution in D_2O.

Total and intermolecular distribution functions, ${}^DG_H(r)$ and ${}^DG_H{}^{inter}(r)$, are shown in Figs. 3a and 3b, respectively. The first peak at $r \approx 1$ Å in ${}^DG_H(r)$ can be assigned to the intramolecular H_M–C interaction. The intramolecular $H_M \cdots N$ and $H_M \cdots C_O$ interactions are considered to be involved in the second peak, located at $r \approx 2.1$ Å. The third peak is attributable to the sum of contributions from the intramolecular $H_M \cdots D_N$ and $H_M \cdots O_C$ interactions. The intermolecular distribution function, ${}^DG_H{}^{inter}(r)$, shown in Fig. 3b, exhibits a considerably broadened feature, implying very weak interaction between the methylene-hydrogen atoms and the nearest neighbor water molecule. Since the present ${}^DG_H{}^{inter}(r)$ function involves contributions from both $H_M \cdots O_W$ and $H_M \cdots H_W$ interactions, details of hydration geometry of the water molecules cannot be obtained from ${}^DG_H{}^{inter}(r)$ alone. Information concerning the partial distribution functions, $a_{H_MO_W}(Q)$ and $a_{H_MH_W}(Q)$, is therefore necessary.

Figure 4 represents the total and intermolecular difference functions, ${}^{0H}\Delta_H(Q)$ and ${}^{0H}\Delta_H{}^{inter}(Q)$, respectively. Although data points are somewhat scattered due to the large incoherent scattering background of H atoms involved in the sample solutions Ⅲ and Ⅳ, the oscillational interference feature can be observed in ${}^{0H}\Delta_H(Q)$ (Fig. 4a). The normalization factor, γ, was calculated to be $\gamma = 0.99 \pm 0.02$, indicating that the data correction and normalization procedures have been adequately carried out. The calculated $I_H{}^{intra}(Q)$ function (Fig. 4b) was then subtracted from ${}^{0H}\Delta_H(Q)$ to obtain the intermolecular difference function, ${}^{0H}\Delta_H{}^{inter}(Q)$, which is shown in Fig. 4c.

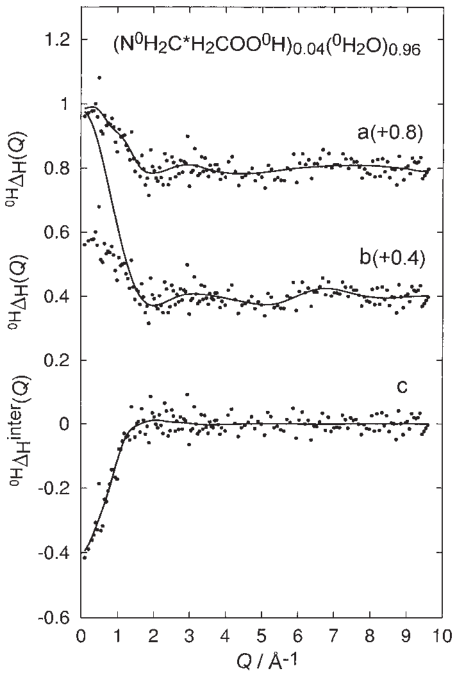

Fig. 4. Same notations as in Fig. 2 except for the aqueous 4 mol% glycine solution in ${}^0H_2O$.

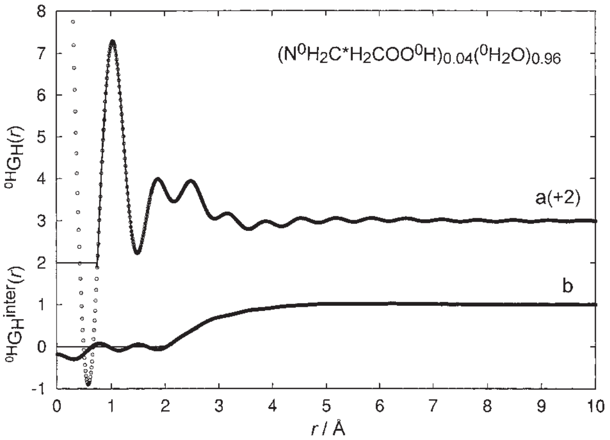

Fig. 5. Same notations as in Fig. 3 except for the aqueous 4 mol% glycine solution in ${}^0H_2O$.

Total and intermolecular distribution functions, ${}^{0H}G_H(r)$ and ${}^{0H}G_H^{inter}(r)$, are represented in Figs. 5a and 5b, respectively. A dominant first peak appearing at $r \approx 1$ Å in the present ${}^{0H}G_H(r)$ function can be assigned to the intramolecular H_M–C_M bond within the glycine molecule. The second and the third peaks at $r \approx 1.9$ and 2.5 Å are attributable to intramolecular $H_M \cdots N + H_M \cdots C_O$ and $H_M \cdots O_C$ interactions, respectively. Periodical features superimposed on ${}^{0H}G_H(r)$ are considered to be termination ripples arising from the relatively small value of Q_{max} ($= 9.6$ Å^{-1}) applied in the Fourier transform. These termination errors are largely suppressed in the intermolecular distribution function, ${}^{0H}G_H^{inter}(r)$, shown in Fig. 5b. Since no contribution from the $H_M \cdots H_W$ pair is involved in the present ${}^{0H}G_H^{inter}(r)$ function, ${}^{0H}G_H^{inter}(r)$ denotes the distribution of the water-oxygen atoms around the methylene-hydrogen atoms of the glycine molecule. The ${}^{0H}G_H^{inter}(r)$ function looks rather featureless, which again implies a very weak hydration nature with regard to the methylene group.

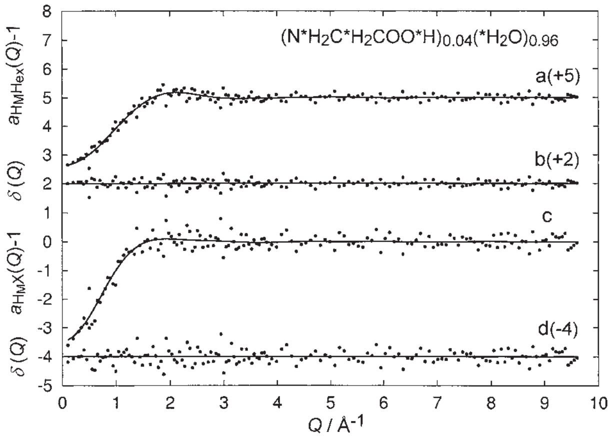

Fig. 6. Dots: Partial structure factors, a) $a_{H_MH_{ex}}(Q)$ and c) $a_{H_MX}(Q)$, observed for the aqueous 4 mol% glycine solution. Solid lines: The best-fit of the calculated interference terms in Eq. 10. The differences between observed and calculated interference functions, $\delta(Q)$, for $a_{H_MH_{ex}}(Q)$ and $a_{H_MX}(Q)$, are given by b) and d), respectively.

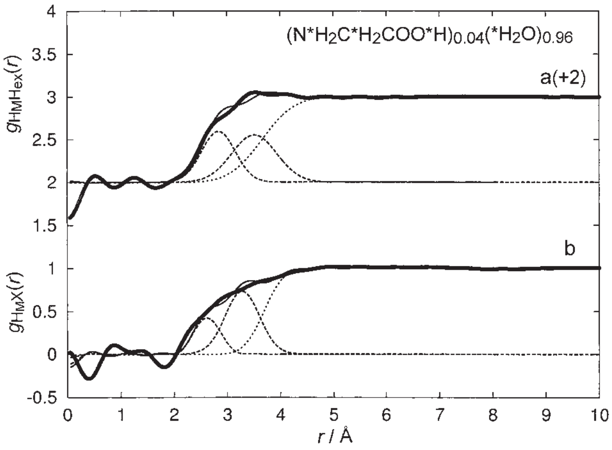

Fig. 7. Thick solid lines: a) $g_{H_MH_{ex}}(r)$ and b) $g_{H_MX}(r)$ observed for the aqueous 4 mol% glycine solution. Thin solid lines: Fourier transform of the best-fit of calculated interference terms shown in Fig. 6. Short- and long-range components are denoted by broken and dotted lines, respectively.

In order to obtain more detailed information concerning the hydration structure around the methylene group, the least squares refinement procedure was applied to the observed $a_{H_MH_{ex}}(Q)$ and $a_{H_MX}(Q)$ partial structure factors derived through Eqs. 7 and 8, respectively. The observed $a_{H_MH_{ex}}(Q)$ and $a_{H_MX}(Q)$ functions are shown in Figs. 6a and 6b, respectively. The corresponding distribution functions, $g_{H_MH_{ex}}(r)$ and $g_{H_MX}(r)$, are represented in Figs. 7a and 7b, respectively. In the preliminary analysis, at least two short-range interactions were found to be necessary to reproduce both the $a_{H_Mj}(Q)$ and $g_{H_Mj}(r)$ (j = H_{ex} and X) functions. In the present analysis, the number of the short-range interactions was assumed to be two.

Table 3. Results of the Least Squares Refinement for the Partial Structure Factor, $a_{\mathrm{H_M H_{ex}}}(Q)$, Observed for Aqueous 4 mol% Glycine Solutions[a)]

Interaction	i···j	r_{ij}/Å	l_{ij}/Å	n_{ij}
Short-range	$\mathrm{H_M \cdots H_W(I)}$[b)]	2.87(1)	0.32(1)	3.1(2)
	$\mathrm{H_M \cdots H_W(II)}$[c)]	3.56(2)	0.42(2)	5.9(3)
		r_0/Å	l_0/Å	
Long-range	$\mathrm{H_M \cdots H}$	3.72(1)	0.48(1)	

a) Estimated standard deviations are given in parentheses. b) The first nearest neighbor $\mathrm{H_M \cdots H_W}$ interaction. c) The second nearest neighbor $\mathrm{H_M \cdots H_W}$ interaction.

Table 4. Results of the Least Squares Refinement for the Partial Structure Factor, $a_{\mathrm{H_M X}}(Q)$, Observed for Aqueous 4 mol% Glycine Solutions[a)]

Interaction	i···j	r_{ij}/Å	l_{ij}/Å	n_{ij}
Short-range	$\mathrm{H_M \cdots O_W(I)}$[b)]	2.64(1)	0.24(1)	0.86(3)
	$\mathrm{H_M \cdots O_W(II)}$[c)]	3.31(1)	0.32(1)	3.10(3)
		r_0/Å	l_0/Å	
Long-range	$\mathrm{H_M \cdots X}$[d)]	3.71(1)	0.30(1)	

a) Estimated standard deviations are given in parantheses. b) The first nearest neighbor $\mathrm{H_M \cdots O_W}$ interaction. c) The second nearest neighbor $\mathrm{H_M \cdots O_W}$ interaction. d) X: O, C, N, and $\mathrm{H_M}$.

The result of the least squares fit for the observed $a_{\mathrm{H_M H_{ex}}}(Q)$ and $a_{\mathrm{H_M X}}(Q)$ functions are shown in Figs. 6a and 6b, respectively. A satisfactory agreement is obtained between the observed and calculated fits over the whole Q-range employed. Final values of all independent parameters are summarized in Tables 3 and 4. The present value of the nearest neighbor $\mathrm{H_M \cdots O_W}$ distance, $r_{\mathrm{H_M O_W}} = 2.64(1)$ Å, is close to the sum of the van der Waals radii of the hydrogen and oxygen atoms, indicating very weak interaction between the methylene-hydrogen atoms and the water molecules within the first hydration shell. The coordination number, $n_{\mathrm{H_M O_W}} = 0.86(3)$, indicates, on the average, ca. two water molecules are involved in the first hydration shell of the methylene group of the glycine molecule. The present value of $n_{\mathrm{H_M O_W}}$ is in good agreement with that reported for the aqueous glycine solutions at high-pH ($n_{\mathrm{H_M O_W}} = 0.81(6)$),[4] which was derived from the least squares fit of the intermolecular difference function, $\Delta_{\mathrm{H}}^{\mathrm{inter}}(Q)$, involving contributions from both $\mathrm{H_M \cdots O_W}$ and $\mathrm{H_M \cdots H_W}$ interactions. In the acidic solution, the value of $n_{\mathrm{H_M \cdots D_2O}}$ was reported to be 0.66(1),[5] suggesting the first hydration shell of the methylene group of the glycine molecule is perturbed by the ionization state of the glycine molecule. The nearest neighbor $\mathrm{H_M \cdots H_W}$ distance was determined to be 2.87(1) Å. The angle between the $\mathrm{H_M \cdots O_W}$ axis and the molecular plane of the water molecule was estimated to be 85(3)°, employing the known molecular geometry of the water molecule in the liquid state ($r_{\mathrm{OH}} = 0.983$ Å and $r_{\mathrm{HH}} = 1.55$ Å).[21,22]

These results indicate that the orientation of water molecules in the first hydration shell of the methylene group of the glycine molecule is not completely random. Recently, the hydration structure around the methyl group of the alanine molecule has been investigated by neutron diffraction with H/D isotopic substitution method.[23] The observed partial distribution functions, $g_{\mathrm{H_M X}}(r)$ and $g_{\mathrm{H_M H_{ex}}}(r)$, exhibited partially resolved nearest neighbor $\mathrm{H_M \cdots O_W}$ ($\mathrm{H_M}$: methyl-hydrogen atom) and $\mathrm{H_M \cdots H_W}$ peaks.[23] These results indicate that water molecules in the first hydration shell of the methyl group assume a preferred orientation. The present results may suggest that the apolar group of the amino acid molecule has some particular hydration structure in aqueous solution.

In our previous neutron diffraction study using the $^{14}\mathrm{N}/^{15}\mathrm{N}$ isotopic substitution method, it was revealed that 3.0(6) water molecules are hydrogen bonded to the amino group of the glycine molecule in neutral aqueous solution.[3] If we assume that the hydration number of the carboxyl group of the glycine molecule is similar to that found for the formate (4.4–4.8)[24] and for the acetate (4.0(1))[25] ions, the total number of water molecules within the first hydration sphere of the glycine molecule can be approximately estimated to be 8–9, which is in good agreement with that observed for the alanine molecule.[23] In the present aqueous 4 mol% glycine solution, ca. 30–40% of the total water molecules are mobilized to form the first hydration shell of the glycine molecules.

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